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Published in:
Investigative ophthalmology & visual science

DOI:
10.1167/iovs.15-17920

Publication date:
2015

A Nonsense Mutation in *FAM161A* Is a Recurrent Founder Allele in Dutch and Belgian Individuals With Autosomal Recessive Retinitis Pigmentosa

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Submitted: August 10, 2015
Accepted: September 30, 2015

Citation: Van Schil K, Klevering BJ, Leroy BP, et al. A nonsense mutation in *FAM161A* is a recurrent founder allele in Dutch and Belgian individuals with autosomal recessive retinitis pigmentosa. *Invest Ophthalmol Vis Sci*. 2015;56:7418–7426. DOI:10.1167/iovs.15-17920

Purpose. To identify mutations in *FAM161A* underlying autosomal recessive retinitis pigmentosa (arRP) in the Dutch and Belgian populations and to investigate whether common *FAM161A*-associated phenotypic features could be identified.

Methods. Homozygosity mapping, amplification-refractory mutation system (ARMS) analysis, and Sanger sequencing were performed to identify mutations in *FAM161A*. Microsatellite and SNP markers were genotyped for haplotype analysis. Patients with biallelic mutations underwent detailed ophthalmologic examinations, including measuring best-corrected visual acuity, extensive fundus photography with reflectance and autofluorescence imaging, and optical coherence tomography.

Results. Homozygosity mapping in 230 Dutch individuals with suspected arRP yielded five individuals with a homozygous region harboring *FAM161A*. Sanger sequencing revealed a homozygous nonsense mutation (c.1309A>T; p.[Arg437*]) in one individual. Subsequent ARMS analysis and Sanger sequencing in Dutch and Belgian arRP patients resulted in the identification of seven additional individuals carrying the p.(Arg437*) mutation, either homozygously or compound heterozygously with another mutation. Haplotype analysis identified a shared haplotype block of 409 kb surrounding the p.(Arg437*) mutation in all patients, suggesting a founder effect. Although the age of onset was variable among patients, all eight developed pronounced outer retinal loss with severe visual field defects and a bull's eye–like maculopathy, followed by loss of central vision within 2 decades after the initial diagnosis in five subjects.

Conclusions. A founder mutation in *FAM161A* p.(Arg437*) underlies approximately 2% of arRP cases in the Dutch and Belgian populations. The age of onset of the retinal dystrophy appears variable, but progression can be steep, with almost complete loss of central vision later in life.

Keywords: *FAM161A*, retinitis pigmentosa, founder mutation, bull's eye–like maculopathy

Retinitis pigmentosa (RP; MIM[268000]) is a set of hereditary retinal dystrophies affecting more than 1 million people worldwide. It is a progressive disease that typically presents with degeneration of the rod photoreceptors, followed by loss of cone photoreceptor function. Most patients experience night blindness as the initial symptom. Subsequently, a gradual constriction of the peripheral visual fields occurs, followed by loss of central vision. The clinical presentation of RP is highly variable and is matched by an impressive genetic heterogeneity: currently, mutations in 55 genes have been implicated in the pathogenesis of autosomal recessive RP (arRP).[1,2] Genes that are mutated in RP encode proteins with diverse functions in multiple cellular processes, including the phototransduction cascade, the visual cycle, cytoskeletal dynamics, regulation of gene transcription, and ciliary function.[2]

In 2010, two back-to-back studies revealed null-mutations in *FAM161A* as a cause of arRP in the Israeli and German population, respectively.[3,4] *FAM161A* encodes a 716-aa protein that localizes to the connecting cilium, the basal body region, and the adjacent centriole in photoreceptor cells.[5,6] The

connecting cilium is a key structure in mediating the high-throughput transport of essential proteins and lipids from the inner segment (IS) to the outer segment (OS). The photoreceptor OS is in fact considered a specialized and modified cilium that is not self-sustaining and relies on the IS for the synthesis of the essential proteins and lipids.[7] FAM161A is a microtubule-associated ciliary protein presumably involved in maintaining microtubule stability. The interaction with other ciliary and centrosomal proteins known to be implicated in retinal dystrophies, like SDCCAG8, CEP290, lebercilin, and POC1B, points to a possible role for FAM161A in transport processes between the IS and OS.[5,6,8] In addition to its ciliary function, a recent study presenting the FAM161A interactome also suggests a role for FAM161A in more general cellular processes, in the Golgi apparatus, centrosome, and/or the microtubule network.[9]

In this study, we aimed to explore the contribution of *FAM161A* mutations to the genetic spectrum of arRP in the Dutch and Belgian populations. A previously described nonsense mutation p.(Arg437*)[3,10–12] was identified in eight individuals, five times in a homozygous state and three times in compound heterozygous state with another protein-truncating mutation. Detailed clinical examinations revealed some common phenotypic features related to *FAM161A*-associated arRP.

Methods

Subjects

This study was approved by the medical ethics committees of the participating centers, and adhered to the tenets of the Declaration of Helsinki. Before this study, patients and their relatives consented to participate in this study, to retrieve the medical records, and to analyze their DNA.

Genetic Evaluation

To identify conspicuous homozygous regions potentially harboring the genetic defects underlying arRP in the Dutch population, we previously performed genome-wide homozygosity mapping in 230 affected individuals from 186 unrelated, mainly nonconsanguineous families using the Affymetrix GeneChip Genome-Wide Human SNP Array 5.0 platform. Homozygous regions were identified using Partek genomic suite software (Partek, St. Louis, MO, USA), as described previously.[13] In patients with homozygous regions encompassing *FAM161A* (NM_001201543.1), all exons and intron-exon boundaries of this gene were analyzed with Sanger sequencing as reported previously.[4] Following the identification of the c.1309A>T; p.(Arg437*) mutation, the presence of this variant was assessed in 100 Belgian and 184 Dutch unrelated individuals affected with suspected autosomal recessive or isolated RP using amplification-refractory mutation system (ARMS) analysis. For this, three different primers were designed: a wild-type (wt) forward primer (F_wt), a mutated forward primer containing the c.1309A>T mutation at the 3′ end (F_mut) and a wt reverse primer (R_wt). This mutation was confirmed by Sanger sequencing in all mutation-positive patients. All heterozygous carriers were screened for a second mutation by amplifying all coding regions of *FAM161A*, followed by Sanger sequencing (BigDye Terminator v3.1 Cycle Sequencing Kit on an ABI 3730XL genetic Analyzer; Applied Biosystems, Foster City, CA, USA). A single heterozygous patient, for whom no second mutated allele was found, was screened for mutations in two *FAM161A*-associated CRX-bound regions (CBRs).[3,14] Furthermore, quantitative PCR (qPCR) analysis on genomic DNA was performed to determine the presence of any coding copy number alterations, as previously described.[15] qBasePlus software (Biogazalle, Zwijnaarde, Belgium) was used for data-analysis[16] and two reference genes were used for normalization of the relative quantities. Two positive controls with known copy number were used as a reference to calculate the copy numbers.[15] Conventional PCR primers were designed using Primer3Plus,[17] qPCR primers using PrimerXL (http://www.primerxl.org/, in the public domain). All primer sequences can be found in Supplementary Table S1. Patient numbering is used throughout the text only for patients having a homozygous (P1-P5) or compound heterozygous *FAM161A* mutation (P6-P8). The patient with only one heterozygous mutation is referred to as P^{het}.

Haplotype Analysis

In total, 19 markers were genotyped (see Supplementary Table S2), 18 of which were single nucleotide polymorphisms (SNPs), and one flanking microsatellite. Five of the 18 SNPs were included because of their presence in the haplotypes of the German patients described by Langmann et al.[3] Thirteen additional tagging SNPs were selected using the QuickSNP software.[18] All primer sequences can be found in Supplementary Table S1.

Clinical Evaluation

We collected the available clinical data from the medical files of all eight patients with two *FAM161A* mutations, and retrospective data on visual acuity were converted into logMAR scores. Some patients were reevaluated after the identification of the causative *FAM161A* mutations. Ophthalmic examination included measurement of best-corrected visual acuity (BCVA) (Early Treatment Diabetic Retinopathy Study charts; Precision Vision, Inc., La Salle, IL, USA), biomicroscopy, ophthalmoscopy, and fundus photography. Additional tests were Goldmann kinetic perimetry and ERG according to the standards of the International Society for Clinical Electrophysiology of Vision (ISCEV).[19] Spectral-domain optical coherence tomography (SD-OCT) and blue-light (488 nm) autofluorescence imaging (BAF; Heidelberg Spectralis HRA+OCT; Heidelberg Engineering, Heidelberg, Germany) was carried out as described previously.[20] As *FAM161A*-related arRP is a ciliopathy and ciliopathies can be quite diverse and involve other organ systems, we used a questionnaire to identify extraocular features, such as nephropathy, polydactyly, intellectual disability, and obesity. P^{het} is not included in the clinical overview given in the Table, due to the lack of clinical information.

Results

Identification of *FAM161A* Mutations

Following genome-wide homozygosity mapping in a large cohort of Dutch RP patients,[13] five patients with homozygous regions of at least 2 Mb encompassing *FAM161A* were analyzed for mutations in this gene. In one individual (P1), a homozygous nonsense mutation was identified, c.1309A>T; p.(Arg437*) (Fig. 1A). This mutation had previously been identified in three German families segregating arRP.[3] To further explore the prevalence of this mutation in the Dutch and Belgian populations, ARMS analysis was performed for this mutation (Fig. 1A). Of 284 patients, 2 additional patients carried the p.(Arg437*) mutation in a homozygous state (P2 and P3), whereas 4 individuals were heterozygous carriers of this mutation. Screening of the coding region revealed a second heterozygous mutation in three of them, that is, P6

Table. Clinical Overview

Patient ID/ Sex/ Origin	*FAM161A* Mutations		Age at Diagnosis/ Recent Exam	History	VA Snellen, logMAR		Refraction		Lens	Ophthalmoscopy	Goldmann Perimetry	Optical Coherence Tomography	Fundus Autofluorescence
	Allele 1	Allele 2			RE	LE	RE	LE					
P1/M/Dutch	c.1309A>T p.(Arg437*)	c.1309A>T p.(Arg437*)	29/43	Night blindness and visual field loss noted in third decade	0.12 (0.9)	0.3 (0.5)*	−6.25 to 2.50 × 25°	−3.00 to 3.00 × 180°	PSC cataract	Moderate pallor optic discs; severely attenuated vessels; mild bull's eye–like lesions; RPE atrophy periphery with intraretinal bone-spicule pigmentations	Constricted up to 5° with small temporal island BE	Preserved RPE and photoreceptors in the macular region	Hyper-autofluorescent ring around the fovea, with hypo-autofluorescent spots in the midperiphery
P2/M/Dutch	c.1309A>T p.(Arg437*)	c.1309A>T p.(Arg437*)	11/65	Night blindness since age 15 y; fast decrease of VA since age 20 y; visual field loss since age 30 y	LP (2.7)	LP (2.7)	−6.00 to 1.50 × 53°	5.5 to 2.00 × 125°	PSC cataract (visually disturbing)	Pale-white optic discs and severely attenuated vessels; preserved narrow ring (RE) or doughnut-shaped area (LE) of retina surrounding the atrophic macula; very severe chorioretinal atrophy with intraretinal bone-spicule pigmentations in periphery	Constricted up to 5° (RE) and 10° (LE) at age 31 y	Severely atrophic retina with a relatively preserved retina with a RPE/choroid band in the area corresponding with the ring that surrounds the macula	Diffuse scleral reflectance with a hypo-autofluorescent ring around the macula
P3/F/Dutch	c.1309A>T p.(Arg437*)	c.1309A>T p.(Arg437*)	25/67	Night blindness and visual field loss since age 25 y; subsequent loss of VA	LP (2.7)	LP (2.7)	−2.50	−2.50	PSC cataract	Pale-white optic disc; severely attenuated vessels; preserved doughnut-shaped area of macula around atrophic fovea; periphery with intraretinal bone-spicule pigmentations	NP	NP	NP
P4/F/Dutch	c.1309A>T p.(Arg437*)	c.1309A>T p.(Arg437*)	41/57	Night blindness and visual field loss noted in third decade	0.7 (0.16)	0.6 (0.2)	-plano-2.00 × 99°	−0.75 to 0.75 × 105°	PSC cataract (visually disturbing)	Mild pallor optic discs; severely attenuated vessels; mild RPE changes perifoveally; round lesions with RPE atrophy periphery with bone-spicule pigmentations	Constricted up to 10° RE and 5–10° LE (at age 53 y)	Preserved RPE and photoreceptors in the macular region	Hyper-autofluorescent ring around the fovea, with hypo-autofluorescent spots in the midperiphery
P5/F/Dutch	c.1309A>T p.(Arg437*)	c.1309A>T p.(Arg437*)	31/52	Night blindness and visual field loss noted in second decade	0.05 (1.3)*	0.5 (0.3)	−0.75 to 2.25 × 86°	−2.00	PSC cataract (visually disturbing)	Mild pallor optic discs; severely attenuated vessels; mild bull's eye–like lesions; epiretinal changes along vascular arcade; round lesions with RPE atrophy periphery with bone-spicule pigmentations	Constricted up to 50–10° BE	Preserved RPE and photoreceptors in the macular region	Hyper-autofluorescent ring around the fovea, with hypo-autofluorescent spots in the posterior pole and midperiphery
P6/M/Dutch	c.1309A>T p.(Arg437*)	c.1501del p.(Cys501Valfs*4)	19/25	Night blindness and visual field loss noted in second decade	0.9 (0.04)	0.9 (0.04)		−0.75 to 0.75 × 105°	PSC cataract (mild)	Mild pallor optic discs; severely attenuated vessels; mild bull's eye–like lesions, wrinkling inner limiting membane; RPE atrophy periphery with bone-spicule pigmentations	Constricted up to 15–20° BE	Preserved RPE and photoreceptors in the macular region	Hyper-autofluorescent ring around the fovea, with hypo-autofluorescent spots in the midperiphery
P7/F/Belgian	c.1309A>T p.(Arg437*)	c.1567C>T p.(Arg523*)	32/43	Night blindness since age 7 y; visual field loss since age 23 y; marked loss of VA since age 40 y	LP (2.7)	LP (2.7)	−2.50 to 3.00 × 45°	−2.25 to 2.50 × 145°	PSC cataract	Waxy optic discs with temporal pallor; severely attenuated retinal vessels; RPE atrophy more pronounced in confluent round flecks around vascular arcades and inferiorly; periphery mixed nummular and bone-spicule pigmentations	Central perception of object V4e in RE; NR in LE	Preserved RPE and photoreceptors immediately inferior to atrophic fovea (RE > LE)	Marked hypo-autofluorescence of area around optic disc & vascular arcades, with nummular patches of hypo-autofluorescence in midperiphery
P8/M/Belgian	c.1309A>T p.(Arg437*)	c.183+1G>T p.(?)	25/62	Night blindness since age 6 y; visual field loss since age 10 y; marked loss of VA since age 38 y	0.05 (1.3)	LP (2.7)	−4.00 to 2.00 × 180°	−4.00	Pseudophakia	Waxy optic discs with temporal pallor; severely attenuated retinal vessels; preserved doughnut-shaped area of macula around atrophic fovea; RPE atrophy more pronounced around vascular arcades periphery with mixed nummular and bone-spicule pigmentations	NR BE	Preserved RPE and photoreceptors in doughnut-shaped concentric circle around atrophic fovea (RE > LE)	Marked hypo-autofluorescence of area around optic disc & vascular arcades, with hyper-autofluorescent ring around fovea

All individuals are unrelated, except P4 and P5, who are sisters. All full field ERG was nonrecordable except for P4, for whom it was not performed. BE, both eyes; CF, counting fingers; F, female; M, male; NP, not performed; NR, nonrecordable; PSC, posterior subcapsular; VF, visual field.

* Amblyopia.

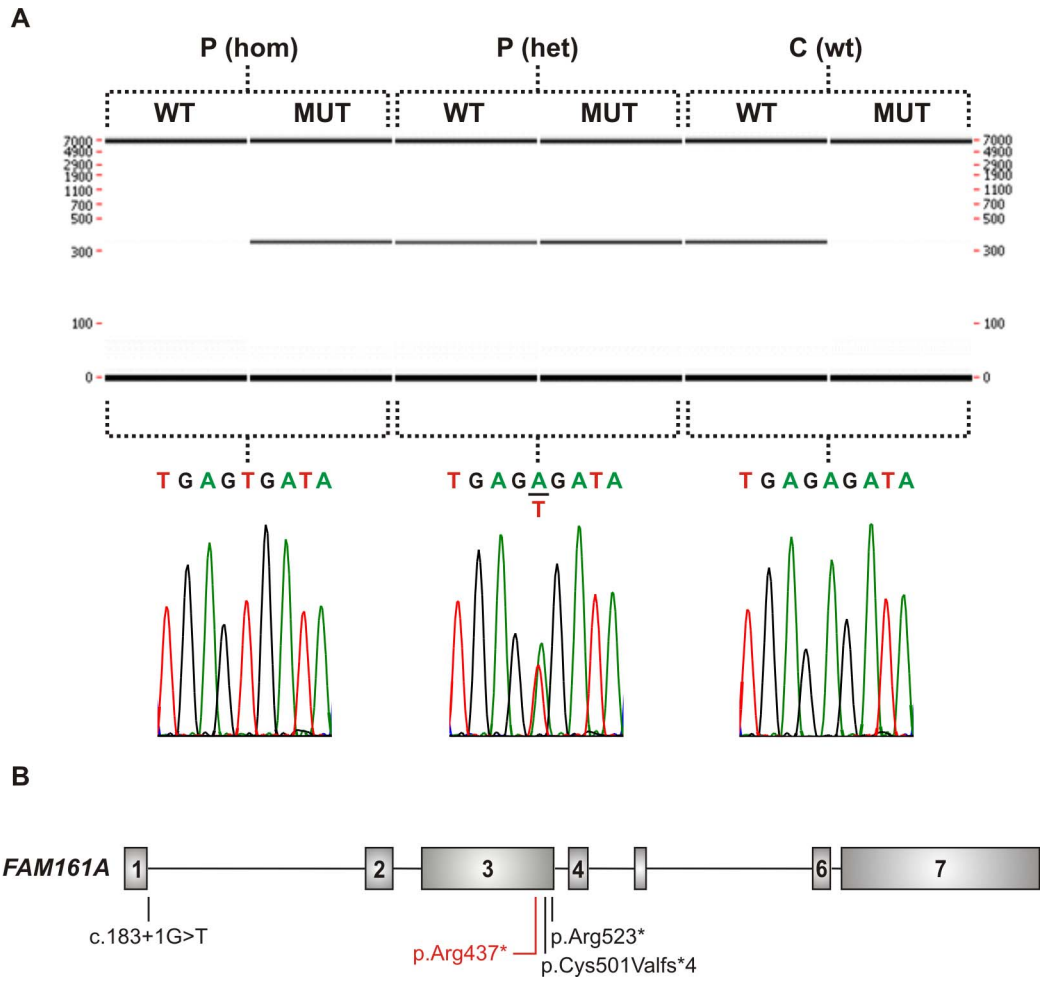

Figure 1. *FAM161A* mutations identified in this study. (**A**) Identification of *FAM161A* founder mutation. Results of the ARMS reaction and subsequent Sanger sequencing analysis in patients homozygous for the c.1309A>T mutation, P (hom), heterozygous patients, P (het) and controls with two wild-type (wt) alleles, C (wt). The ARMS analysis consisted of two PCR reactions for every patient, one with a forward primer matching the wt allele (WT lane), the other one with a primer matching the mutated allele (MUT lane). P (hom) will only have amplification in the MUT lane, P (het) in both lanes and C (wt) only in the WT lane. Every mutation detected with the ARMS reaction was confirmed by Sanger sequencing. (**B**) Overview of *FAM161A* mutations identified in this study. The p.(Arg437*) founder mutation (*red*) is located in the third and largest exon of *FAM161A*. Presence of this mutation has been demonstrated in a total of nine Dutch and Belgian patients, of which five were homozygous for this mutation. In three heterozygous patients, a second mutation has been identified (*black*): c.183+1G>T, disrupting the 5′ splice site of the first intron; p.(Cys501Valfs*4), a frameshift mutation leading to the incorporation of a premature stop codon; p.(Arg523*), a second nonsense mutation further downstream in the third exon. In one patient (P^{het}) no second mutation was identified.

carried a 1-bp deletion leading to a frameshift and the incorporation of a premature stop codon (c.1501del, p.[Cys501Valfs*4]), P7 carried a second nonsense mutation (c.1567C>T, p.[Arg523*]), and P8 carried a mutation abolishing the 5′ splice site of the first intron (c.183+1G>T). No second exonic mutation was detected in the fourth heterozygous carrier P^{het}. Copy number variations of the coding region of *FAM161A* as a potential second mutation were excluded by genomic qPCR analysis. Since the expression of *FAM161A* is known to be strictly regulated by the retinal transcription factor CRX, reflected by the association of the gene with two evolutionarily conserved upstream and intronic CBRs respectively (Supplementary Fig. S1), we hypothesized that disruption of the binding sequence for CRX could alter the regulation of this gene.[14] Hence, both CBRs were sequenced for this individual but no sequence variation was identified. Interestingly, P^{het} had two nieces with RP, whose DNA was not present in the initial cohort. Sanger sequencing subsequently revealed the p.(Arg437*) mutation to be present in homozygous state in these two sisters (P4 and P5). Of note, both sisters also carry a heterozygous mutation in *RPE65* (c.11+5G>A) that is recurrently present in the village where they live. In total, eight individuals were identified with biallelic mutations in *FAM161A*, with the p.(Arg437*) representing at least one of the two alleles in all cases. All *FAM161A* mutations identified in this study are depicted in Figure 1B.

The p.(Arg437*) Mutation in *FAM161A* Is a Founder Mutation

Given the high prevalence of the p.(Arg437*) mutation in the Dutch and Belgian cohorts, on top of the fact that this mutation had also been described in the German population,[3] we hypothesized that this allele may represent a founder mutation. To assess this, haplotype analysis was performed in all patients carrying this mutation by genotyping the 5 SNPs reported by Langmann et al.[3] and extended by 13 additional SNPs. The haplotype was compared with the previously reported haplotype of the German patients. All individuals homozygous for the p.(Arg437*) mutation (P1 to P5), were also homozygous

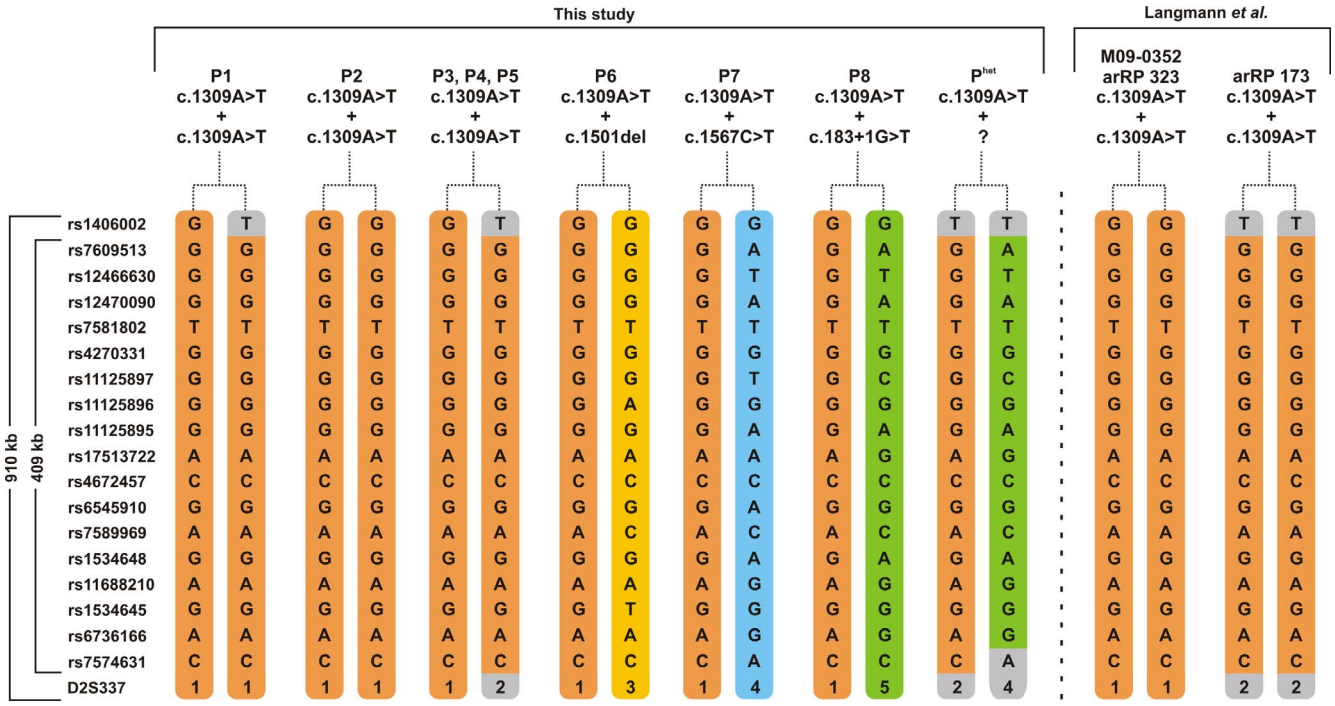

Figure 2. Haplotype analysis of *FAM161A* locus in Dutch, Belgian, and German patients. Eighteen SNPs and one microsatellite marker spread over a region of 910 kb (chr2: 61,669,931 [D2S337]–62,579,956 [rs1406002] [hg19]) were used to perform haplotype analysis in the eight individuals carrying the putative p.(Arg437*) founder mutation. An identical (*orange*) haplotype block of 409 kb (chr2: 61,825,142 [rs7574631]–62,234,345 [rs7609513] [hg19]) linked to disease could be identified in each of the patients. As expected, the five homozygous patients (P1–P5) described in this study and the three homozygous German patients described by Langmann et al.[3] carried two copies of this disease haplotype block, whereas the three heterozygous patients had only one copy. Remarkably, P8 and P^{het} appear to share the same haplotype combination, while the splice site mutation identified in P8 was absent in P^{het}.

for a number of investigated SNPs surrounding the mutation, allowing construction of the haplotype carrying the nonsense mutation. The three individuals who carried compound heterozygous mutations (P6 to P8) also carried one of these haplotypes, together with a different haplotype harboring the second mutation. In addition, the disease haplotype identified in this study corresponded to the haplotype of the German patients who were homozygous for the p.(Arg437*) mutation (Fig. 2). The 17 SNPs (rs7609513–rs7574631) that together define the shared haplotype block are spread out over a region of 409 kb, delimited by recombination events at flanking SNP rs1406002 and flanking microsatellite D2S337, pointing to a maximal length of 910 kb for the common haplotype and further emphasizing that the p.(Arg437*) mutation represents a founder allele. Not taking into account the initial patient P1 included in the homozygosity study, P^{het} and her two nieces P4 and P5, we identified the founder mutation either in homozygous or compound heterozygous state in 5 out of 284 patients, corresponding to a prevalence of approximately 2% in the Dutch and Belgian populations.

Clinical Evaluation

Eight affected individuals from seven families were included in the study and an overview of the mutations and the clinical data is presented in the Table and Figure 3. The p.(Arg437*) mutation was found in a homozygous state in five patients of four families, albeit that there was no reported parental consanguinity but for P8, whereas P4 and P5 originate from a genetic isolate.

The initial symptom was night blindness in all eight affected individuals, but the age at which it was noted varied from 6 to 25 years. In patient P2, the diagnosis was established during a routine ophthalmic checkup at the age of 11 years; it took another 4 years before this patient became aware of night blindness. Subjects P7 and P8 experienced night blindness from the age of 7 and 6, but were only diagnosed with RP at the age of 32 and 25, respectively, indicating that the night blindness did not have an impact on their daily life and was not a reason to consult an ophthalmologist at an earlier age.

The nyctalopia was followed by progressive concentric constriction of the peripheral visual fields in all patients, and deterioration of central vision.

Of six patients, follow-up data on BCVA were available and are displayed in Figure 4. These data show a loss of central vision after the age of 25 and legal blindness (visual acuity [VA] $<$ 20/400, logMAR 1.3) in the sixth decade in P2 and P3. The other four individuals retained good central vision ($\geq$20/40, logMAR 0.3) even within the sixth decade (P4 and P5) but with small visual fields. No extensive follow-up data were available on P7 and P8, but BCVAs in the better eye were light perception (LP) with localization and decimal BCVA of 0.05 (logMAR 1.3) at ages 43 and 63 years, respectively, with self-reported rapid deterioration of central vision at ages 30 and 36, respectively.

All patients displayed a mild to moderate myopia; anisometropia was the cause of amblyopia in patient P1. Posterior subcapsular cataracts were present in all eight patients. These typical cataracts developed from the third decade; patient P8 underwent cataract extractions at the ages of 57 left eye (LE) and 58 right eye (RE).

Ophthalmoscopy revealed the classic symptoms of RP consisting of waxy pallor of the optic discs and in advanced cases pale-white discs, attenuated retinal vessels, and atrophy of the RPE and choriocapillaris in the midperiphery with intraretinal spicular pigmentation in all eight, combined with

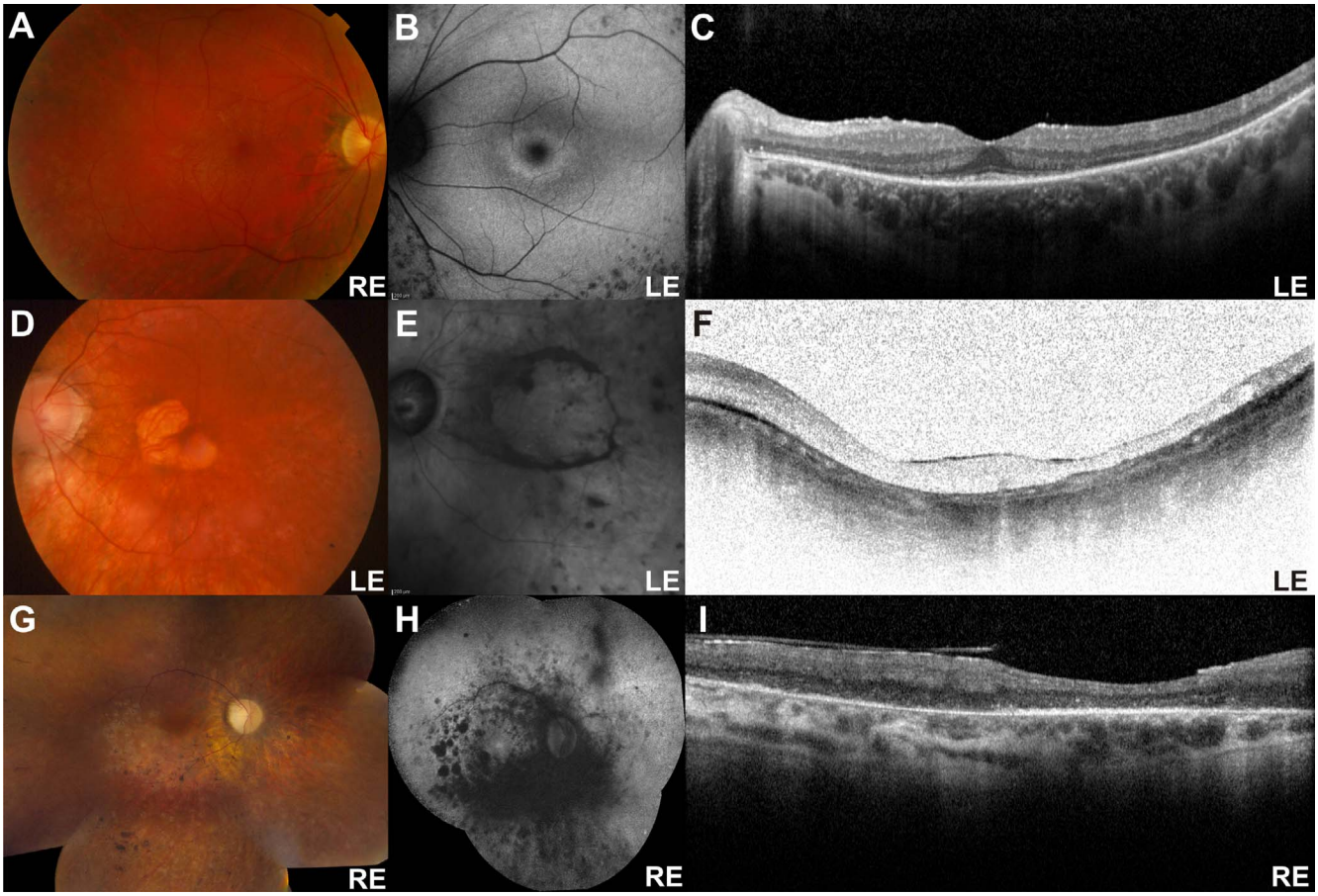

Figure 3. Clinical overview. (**A**–**C**) Images of P6, taken at the age of 25 years. (**A**) Fundus photograph of the RE showing minimal optic disc pallor, attenuated vessels, wrinkling of the inner limiting membrane, mild RPE alterations around the fovea, and atrophy in the midperiphery. (**B**) Fundus autofluorescence of the LE showing a hyperautofluorescent ring around the fovea and hypoautofluorescent patches in the midperiphery. (**C**) Optical coherence tomography of the LE showing intact IS/OS junctions at the macula. (**D**–**F**) Images of P2 at age 62. (**D**) Fundus photograph of the LE at the age of 32 years showing mild pallor of the optic disc, attenuated vessels, distinct RPE atrophy around the fovea, atrophy in the midperiphery with bone-spicule pigmentations. (**E**) Infrared image of the LE at the age of 65 years showing a preserved rim of RPE around the macula with complete atrophy of the fovea. (**F**) Optical coherence tomography of the LE at the age of 65 years showing severely atrophic retina with a relatively preserved retina with a RPE/choroid band in the area corresponding with the ring that surrounds the macula. (**G**–**I**) Images of P7, taken at the age of 43 years. (**G**) Composite fundus photograph of RE showing waxy optic discs with temporal pallor, severely attenuated retinal vessels, RPE atrophy more pronounced in confluent round flecks around vascular arcades and inferiorly, and mixed nummular and bone-spicule pigmentations in the periphery. (**H**) Fundus autofluorescence of the RE showing marked hypoautofluorescence of area around optic disc and vascular arcades, with nummular patches of hypoautofluorescence in midperiphery. (**I**) Optical coherence tomography of the RE showing preserved RPE and photoreceptors immediately inferior to atrophic fovea.

deep intraretinal nummular pigmentations in two (P7 and P8). In four subjects (P1, P4, P5, and P6), a bull's eye–like maculopathy consisting of mild RPE alterations surrounding the fovea was documented at some stage of the disease. In patient P6, this lesion became apparent at age 24. In the three older patients (P2, P3, and P8), a narrow ring of recognizable retinal tissue that surrounds the completely atrophic macular center (Fig. 3) was observed, respectively at age 62, 65, and 67. The retinal tissue peripheral to this ring is also severely atrophic with disseminated, irregular pigmentary deposits. In patients P2 and P3, central ring-like depigmentation or bull's eye–like macular lesions were seen at ages 44 and 53, respectively. Patient P7 displayed a relatively preserved fovea but without function.

Electroretinogram responses could not be elicited in any of the patients, not even at the early stages.

The BAF images on recent examination revealed a hyperautofluorescent ring around the fovea (Fig. 3) in patients P1, P4, P5, and P6. In patients P2 and P8, hyperautofluorescence in accordance with the doughnut-shaped preservation of the RPE was seen. In patients P1, P4, P5, and P6 IS and OS junctions were intact on OCT at the macular region, whereas they were absent in the other three patients tested.

Although one of the patients (P1) was treated for hypertension and diabetes, we did not detect any extraocular features that were suggestive for syndromic RP.

Discussion

Genetic evaluation of *FAM161A* in a Dutch and Belgian cohort of genetically unsolved arRP patients, revealed eight individuals with biallelic *FAM161A* mutations. All individuals carried at least one c.1309A>T, p.(Arg437*) nonsense mutation; five were homozygous, whereas three were compound heterozygous in conjunction with a second protein-truncating allele. In addition, in one affected heterozygous individual related to two affected homozygous individuals, no second *FAM161A* mutation could be found despite copy number screening and evaluation of retina-specific CBRs. However, mutations in

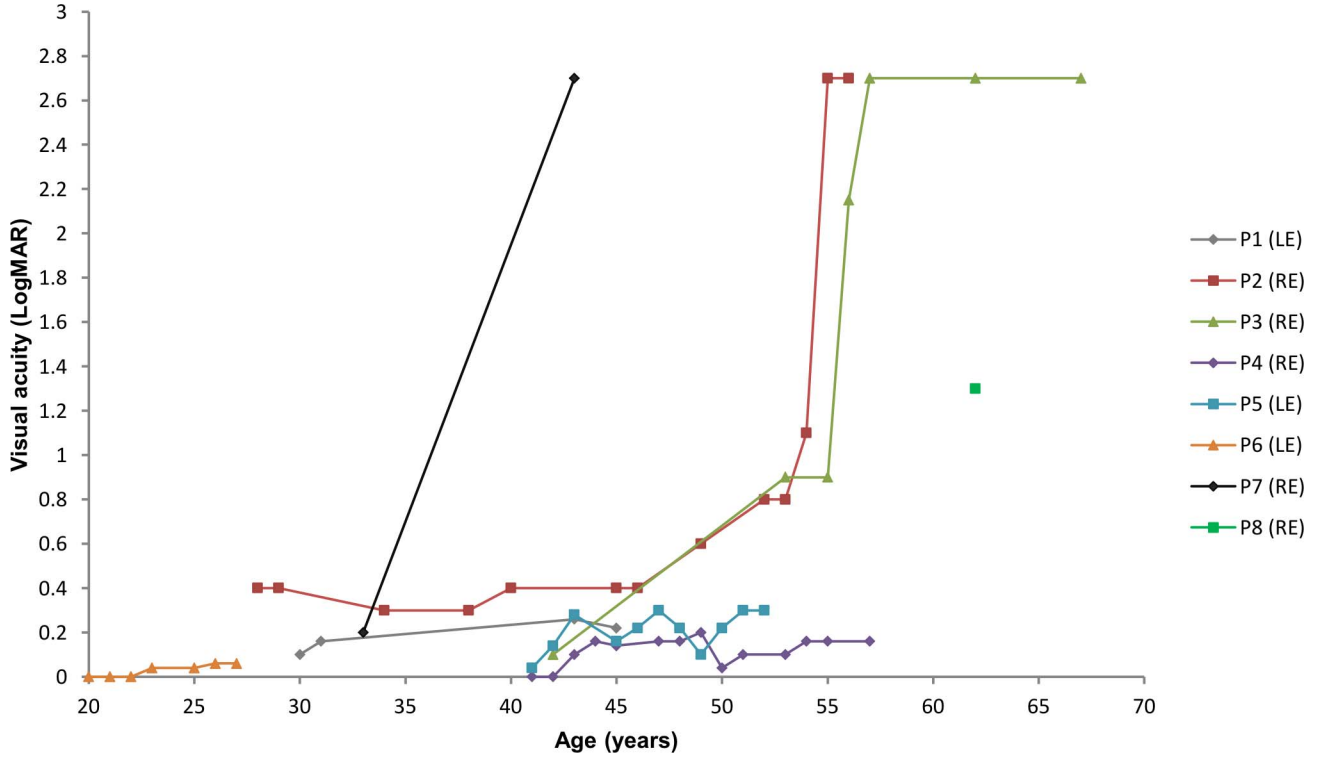

Figure 4. Evolution of VA in patients with *FAM161A* mutations. Graph showing the evolution of BCVAs expressed in logMAR (*y*-axis) over time (age, *x*-axis) for six patients (P1–P6) carrying mutations in the *FAM161A* gene. For P7 and P8, no extensive follow-up data were available, only showing the VA at two and one time points, respectively. Snellen VA was transformed into logMAR for visualization purposes. A logMAR value of 1.9 was assigned to counting fingers (CF), 2.3 to hand movements (HM), and 2.7 to LP. When the VA differed in both eyes, the eye with the best VA at onset was used. When the VA at onset was identical, the recordings of the RE were taken.

noncoding regions of *FAM161A* such as deep intronic mutations, or involvement of mutations in a different retinal dystrophy gene cannot be excluded. Haplotype analysis revealed that the p.(Arg437*) mutation represents a founder allele present in the Dutch, Belgian, and German population.

Following homozygosity mapping, five patients from our cohort showed a significant homozygous region encompassing *FAM161A*, but only one of these carried a homozygous *FAM161A* mutation p.(Arg437*). Yet, this mutation was detected homozygously in four other patients from our cohort. As it appeared, these patients were either not analyzed by homozygosity mapping (P4 and P5) or the homozygous region surrounding the p.(Arg437*) mutation was below the threshold of 250 consecutive homozygous SNPs that was used to identify significant homozygous regions.[13] The relatively small size of the common haplotype block identified in all mutation carriers, including the previously described German patients,[3] supports the hypothesis that this mutation is an ancestral allele that has spread over northwest Europe, explaining the relatively high prevalence of this mutation in our arRP cohorts. However, the disease haplotype described by Rose et al.[12] in British patients with the p.(Arg437*) mutation is different, indicating that this mutation has arisen de novo in at least two different European populations.

Despite a number of recent studies, the exact function of FAM161A is not completely understood. In gene trapped *Fam161a* mice, it was shown that Fam161a is located in the cilia of rod and cone photoreceptors and that this protein is vital for the integrity of the connecting cilium. The structural abnormalities in these *Fam161a*$^{GT/GT}$ mice implicate a crucial role for this protein in the structural composition, maintenance, and function of the connecting cilium; the latter was also demonstrated by the misrouting of the cargo proteins opsin and rds/peripherin.[21] In our study, as well as in previously published work, the vast majority of *FAM161A* alleles are protein-truncating mutations that are predicted to completely abolish the function of the FAM161A protein, whereas only two missense mutations have been reported so far.[10,22] Hence, impaired transport of proteins essential for phototransduction in the OS of photoreceptor cells is the most likely molecular mechanism underlying *FAM161A*-associated arRP.

From a clinical point-of-view, the limited number of previous studies dealing with *FAM161A* mutations has shown a wide range of disease severity,[3,4,12,23,24] although only two reports focus on the phenotype.[12,24] As in other studies, the age of onset of *FAM161A*-associated RP in our patient cohort was variable, ranging from the first to the third decade of life. Most patients with *FAM161A*-associated RP display lens opacities and a mild to moderate myopia. This applies to the Dutch/Belgian patients in this study, but was also mentioned by others.[4,23] However, it is not a finding limited to RP caused by mutations in *FAM161A*.

Despite clinical variability in disease severity, age of onset, and progression, a specific finding in the current study was the very distinct "ring" or "doughnut" of relatively preserved retinal tissue surrounding the macula. This phenomenon was present in three older patients in their seventh decade. The fact that two of these patients had documented bull's eye–like lesions in their fourth and fifth decades, as well as the presence of bull's eye–like maculopathy in two younger patients (P1 and P6) seems to suggest that these phenotypes may be sequential. Although the clinical description is often limited, bull's eye–like patterns can be observed in the fundus photographs of some *FAM161A*-RP patients in other studies.[4,12,24] One of the patients in the report by Bandah-Rozenfeld et al.[4] shows a

fundus autofluorescence image that may even be indicative of a ring of preserved retinal tissue around the macular center.

It would be interesting to investigate whether the presence of a bull's eye–like maculopathy precedes atrophy of the posterior pole, and would thereby act as a negative prognostic factor for VA in *FAM161A*-related RP. Or, as suggested by Rose et al.,[12] that there might be two different phenotypes as observed by fundus autofluorescence pattern.

Progression of the *FAM161A* RP phenotype results in legal blindness in our older patients. This bleak prognosis for VA later in life was also observed by Langmann et al.,[3] but in a recent report by Duncan et al.,[24] VA appeared remarkably well preserved, even in older patients. The notion in the latter study that foveal cones are preserved until the late stages of disease progression was therefore not corroborated by our study, adding to the evidence that this phenotype is indeed highly variable. *FAM161A*-related arRP is a ciliopathy and the phenotypes of this group of disorders can be quite diverse and known to involve other organ systems. We used a questionnaire to identify extraocular features, but except for one patient known with diabetes and hypertension, none of the patients show such associations. Obviously, this does not allow us to entirely and reliably exclude the presence of syndromal abnormalities that can only be brought to light with additional investigations, for instance blood tests or renal ultrasonography in case of renal developmental abnormalities.

In conclusion, we have identified a founder mutation in *FAM161A* underlying visual impairment in approximately 2% of Dutch and Belgian arRP patients. The phenotype of the Dutch/Belgian *FAM161A*-related retinal dystrophy is characterized by a severe chorioretinal atrophy that involves the posterior pole in the later stages, resulting in a very low BCVA that is often limited to LP. A bull's eye pattern was present in most of the patients in the early to midphase of the disease. This maculopathy may well progress to complete central chorioretinal atrophy with the exception of a thin "ring" or "doughnut" shape of relatively preserved retinal tissue surrounding the posterior pole.

Acknowledgments

We gratefully acknowledge Saskia van der Velde-Visser, Willy Verhoef-Thuis, Marlie Jacobs-Camps, and Lonneke Duijkers for technical assistance. We thank our German colleagues Thomas Langmann, Heidi Stöhr, Bernhard Weber, and Andreas Gal for kindly sharing the DNA samples of the German patients previously reported by Langmann et al.[3] Finally, we thank Ramon van Huet for providing visual acuity data of the patients from Nijmegen.

Supported by the Netherlands Organization for Scientific Research (TOP-Grant 91209047), the Algemene Nederlandse Vereniging ter Voorkoming van Blindheid, the Gelderse Blinden Stichting, the Landelijke Stichting voor Blinden en Slechtzienden, the Macula Degeneratie Fonds, the Rotterdamse Stichting Blindenbelangen, and the Stichting Blinden-Penning (FPMC and AidH); the SWOO Flieringa Foundation (LIvdB); and grants from Belspo (IAP Project P7/43), Belgian Medical Genomics Initiative (EDB), and Funds for Research in Ophthalmology (KVS). KVS is doctoral fellow from the Institute for Innovation by Science and Technology. EDB and BPL are senior clinical investigators of the Research Foundation-Flanders (FWO).

Disclosure: **K. Van Schil**, None; **B.J. Klevering**, None; **B.P. Leroy**, None; **J.W.R. Pott**, None; **D. Bandah-Rozenfeld**, None; **M.N. Zonneveld-Vrieling**, None; **D. Sharon**, None; **A.I. den Hollander**, None; **F.P.M. Cremers**, None; **E. De Baere**, None; **R.W.J. Collin**, None; **L.I. van den Born**, None

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